Mini-prep kit – plasmid extraction from bacteria cells

You can extract high/low copy number plasmid out of bacteria cells with this kit.

If you need to keep the starter sterile use sterile conditions.

High Copy Number Protocol

Harvesting -

1. Place **2 mL** of bacterial culture into a 2 mL microcentrifuge tube.
2. Centrifuge for 1 min at full speed (table microcentrifuge) and discard supernatant.
3. Repeat steps 1-2 (total of 4 mL).

Resuspension -

4. Add 200 µL of PD1 Buffer (RNase A added) and pipette (avoid bubbles).

Lysis -

5. Add 200 µL of PD2 Buffer and mix gently by inverting the tube 10 times. **Do not vortex**, avoid shearing genomic DNA.
6. Allow mixture to stand for 2 minutes at room temperature until lysate clears.

Neutralization -

7. Add 300 µL of PD3 Buffer and mix immediately by inverting the tube 10 times.
   Do not vortex.
8. Centrifuge for 2 min at 13,000 rpm (table microcentrifuge).

DNA Binding -

9. Place a PD Column in a 2 mL Collection Tube (provided in the kit), mark with plasmid name.
10. Pour the clear lysate (supernatant) from Step 7 to the PD Column.
11. Centrifuge at 13,000 rpm for 30 seconds.
12. Discard the flow-through and return the PD Column to the 2 mL Collection Tube.

Wash -

13. Add 400 µL of W1 Buffer in the PD Column.
14. Centrifuge at 13,000 rpm for 30 seconds.
15. Discard the flow-through and return the PD Column to the 2 mL Collection Tube.
16. Add 600 µL of Wash Buffer (ethanol added) to PD Column.
17. Centrifuge at 13,000 rpm for 30 Seconds.

18. Discard the flow-through and return the PD Column to the 2 mL Collection Tube.
19. Centrifuge again for 3 min at full speed to dry the column matrix. If the column matrix is not dry, repeat centrifuge.

DNA Elution -

20. Transfer the dried PD Column to a clean 1.5ml microcentrifuge tube (not provided), mark it properly.
21. Add 50µL DDW directly onto the centre of the membrane. Avoid residual buffer adhering to the wall of the column.
22. Allow to stand for 2 min until the liquid is absorbed.
23. Centrifuge for 2 min at 13,000 rpm to elute plasmid DNA.
24. After plasmid purification is complete measure the concentration in the Nano-Drop.
25. Plasmid can be stored at -20°C.